THE MAINTENANCE OF A NORMAL PLASMA PROTEIN CONCENTRATION IN SPITE OF REPEATED PROTEIN LOSS BY BLEEDING

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Two characteristic features of nephritis of the degenerative type are pronounced albuminuria and lowered plasma proteins. The simplest explanation of the plasma protein depletion is, obviously, that regeneration is slow and is unable to keep pace with the albuminuria. The adequacy of this theory could be easily tested if the maximum rate of plasma protein regeneration in normal individuals could be determined. This has not been accomplished in man but has been done in animals.

Kerr, Hurwitz and Whipple (1) studied the regeneration in dogs by noting the time taken for the normal level to be regained after a marked initial depletion. They reduced the protein by the method of plasmapheresis, that is, by the withdrawal of large quantities of blood, followed by the injection of the red cells suspended in Locke's solution after the removal of the plasma. In most of their experiments, over 50 per cent of the total plasma protein was removed and they found that from 7 to 14 days were required for the replacement of the lost protein. Regeneration was only slightly more rapid on a high protein diet than on starvation but was considerably reduced when the liver was damaged. They did not express their results in actual amounts of protein regenerated, but from their protocols, approximate calculations can be made. The highest rate of regeneration in their experiments was about 0.15 gm. per kilo per day. They concluded that the regeneration of plasma protein is a slow process.

Smith, Belt and Whipple (2) repeated this work using a modified bleeding technic which permitted a protein reduction to about one-third of the normal. They studied the curve of regeneration and found an immediate rise within 15 minutes of about 0.5 per cent and a return to normal in from 2 to 7 days. The rates of regeneration were much greater than those observed by Kerr, Hurwitz and Whipple. The average daily regeneration in nine experiments was 0.42 gm. per kilo per day, and the highest, 0.89 gm. per kilo per day.

Schultz, Swanson and Ziegler (3) used the method of Kerr, Hurwitz and Whipple

to study the rate of regeneration of the various protein fractions, but as they published no protocols, the exact rate of regeneration that they observed cannot be determined.

Leiter (4–6), Barker and Kirk (7) and Shelburne and Egloff (8) used the method of plasmapheresis to lower plasma proteins in the study of edema but did not directly measure the rate of regeneration.

In all the previous work regeneration has been studied when the plasma proteins were reduced to a very low level. We have found no record of any experiments designed to determine the maximum amount of protein that can be removed daily over a considerable period of time without lowering the protein level in the blood. The object of the present experiment was to determine this quantity in dogs.

Experimental Method

A series of normal dogs of approximately the same size were taken and varying quantities of blood were removed daily by aspiration from a jugular vein, the amounts removed being kept as nearly constant as possible in each individual dog. Dry sodium citrate was used as an anticoagulant, the blood was centrifuged, the plasma was drawn off, measured and replaced with Locke's solution, after which the cells were kept in an ice box to be reinjected the following day after the next bleeding. The total plasma proteins were measured gravimetrically after coagulation with heat at a pH of 4.9. The method is similar to that of Starlinger and Hartl (9) except that a half normal acetate buffer is used instead of N/50 acetic acid, and filtration is carried out on asbestos filters instead of filter papers, the rapid absorption of water by a dried filter paper making accurate weighing on an analytical balance impossible.

The procedure used is as follows:

Asbestos filters are prepared using thoroughly washed asbestos on Jena glass filters (1 G 3). These are dried to a constant weight in an incubator at 105°C. Complete drying is usually accomplished in 24 hours. All filters are kept in a desiccator over P_2O_5 when not in the incubator.

Blood is taken using dry sodium citrate as an anticoagulant and the plasma is drawn off after centrifuging. 1 cc. of plasma and 5 cc. of half normal sodium acetate acetic acid buffer with a pH of about 4.9 are placed in a test-tube and immersed in boiling water for 15 minutes. The contents of the tube are then filtered through a weighed asbestos filter with suction, care being taken to transfer all the precipitate to the filter. After the filtrate has been completely drawn through, the filter is washed twice with about 10 cc. of distilled water, twice with 95 per cent alcohol, twice with ether and finally twice with boiling absolute alcohol. The filter is then dried to constant weight at 105°C. This is usually accomplished

in 24 hours, but occasionally 48 hours is required. The number of milligrams of protein on the filter divided by 10 gives the plasma protein in per cent.

The determination of protein gravimetrically seems more satisfactory than by any of the indirect methods of estimation. In the present method there are three principal sources of error. First, there is the loss of protein by incomplete coagulation or faulty filtration, second, the inclusion in the precipitate of salts, and third, the inclusion of non-protein organic substances, principally fat. We have attempted to eliminate these possible sources of error and feel that the method is accurate to within 0.3 gm. per cent or about 5 per cent.

TABLE I

Quantities of Protein Lost in the Filtrate and Wash Water

No.	N in filtrate	Non-protein nitrogen	Protein N in filtrate	Protein in filtrate	Protein in ppt.	Loss
	mg.	*mg./cc.*	*mg.*	*mg.*	*mg.*	*per cent*
1	0.21		0.21	1.3	63	2.1
2	0.62	0.35	0.27	1.7	55	3.1
3	0	0.35	0	0	55	0
4	0.39	0.35	0.04	0.25	55	0.5
5	0.50	0.35	0.15	0.9	55	1.6
6	0.52	0.32	0.20	1.2	54	2.2
7	0.52	0.32	0.20	1.2	54	2.2
8	0.25	0.24	0.01	0.06	57	0.1
9	0.26	0.24	0.02	0.12	57	0.2
10	0.32	0.24	0.12	0.8	57	1.4
11	0.23	0.24	0	0	57	0
12	0.60	0.36	0.24	1.5	61	2.5
13	0.55	0.36	0.19	1.2	61	2.0
14	0.45	0.36	0.09	0.6	61	1.0
15	0.35	0.36	0	0	61	0
16	0.42	0.35	0.07	0.4	64	0.6
17	0.42	0.35	0.07	0.4	64	0.6
18	0.47	0.35	0.12	0.8	64	1.2
19	1.14	0.35	0.79	4.9	64	7.6

Table I shows the results of a series of nitrogen determinations by the Kjeldahl method on the filtrates and washings of a number of determinations. The protein lost is calculated by multiplying the total nitrogen content of the filtrate less the non-protein nitrogen in the sample of plasma by 6.25. In 18 of the 19 observations, the percentage loss of protein was less than 3 per cent and in one 7.6 per cent. There is, therefore, no greater loss of protein in the filtrate than the limits of error of the method.

The possible inclusion of inorganic salts was tested by ignition after protein determination, using asbestos on Gooch crucibles instead of glass filters. The

final weights checked in each instance to within 1 per cent of the original weight of the filter. The results of these determinations are shown in Table II.

The inclusion of fat is more difficult to detect and we have made no accurate measurements but have relied upon washing with alcohol, ether and hot absolute alcohol to completely remove fat. Repeated washings with alcohol and ether do not change the weights of the filters and precipitates.

The results of this method are consistent and checks on duplicate determinations are easily obtained to within 0.3 gm. per cent. Most of the observations to

TABLE II

Results of Ignition of the Filter after Protein Determination

Weight after 1st ignition	Weight after 2nd ignition	Weight after 3rd ignition following protein determination
gm.	*gm.*	*gm.*
18.8481	18.8472	18.8467
17.4932	17.4924	17.4921
17.8934	17.8924	17.8925
18.4737	18.4733	18.4729
17.6904	17.6904	17.6899
15.6526	15.6520	15.6517

TABLE III

Agreement of Duplicate Determinations on the Same Sample

No.	1	2	No.	1	2
1	5.54	5.45	7	7.24	7.30
2	5.54	5.25	8	6.41	6.47
3	5.82	5.76	9	7.21	7.09
4	6.49	6.25	10	5.04	5.01
5	5.44	5.47	11	5.20	5.14
6	5.74	5.64	12	6.89	6.99

be recorded here are based on single determinations but no great discrepancies were noted from day to day. In a few instances, duplicate determinations were done and the agreement is shown in Table III.

In no case did duplicate determinations fail to agree except when they were done by different modifications of the method before the procedure described above was adopted.

The accuracy of the method for low serum protein values was demonstrated by measuring the protein content of a series of dilutions of normal plasma. 1 cc. was used in each case and was made up by adding to the amount of plasma shown

in the table, sufficient water to make 1 cc. The results are shown in Table IV and when the observed protein values are compared to the average of all the determinations, an error of less than 3 per cent is observed throughout the entire range except for the determination in which only 0.1 cc. of plasma was used.

TABLE IV

Accuracy of the Method over a Range of Plasma Protein from 0.6 to 7.0 Gm. Per Cent

Plasma	Observed protein	Average	Difference	Error
cc.	*per cent*	*per cent*	*per cent*	*per cent*
0.1	0.65	0.69	0.04	5.8
0.2	1.34	1.38	0.04	2.9
0.3	2.11	2.07	0.04	1.9
0.4	2.75	2.76	0.01	0.4
0.5	3.54	3.45	0.09	2.6
0.6	4.18	4.14	0.04	1.0
0.7	4.74	4.83	0.09	1.9
0.8	5.55	5.52	0.03	0.5
0.9	6.32	6.21	0.11	1.8
1.0	7.04	6.90	0.14	2.0

TABLE V

Agreement between the Method Here Described and the Kjeldahl Method

No.	Protein, gravimetric	Protein, Kjeldahl	Difference	Error (Kjeldahl as standard)
	per cent	*per cent*	*per cent*	*per cent*
1	7.04	7.19	0.15	2.1
2	5.42	5.40	0.02	0.4
3	5.62	5.90	0.28	4.7
4	5.64	5.60	0.04	0.7
5	5.79	5.50	0.29	5.3
6	6.37	5.80	0.57	9.8
7	5.55	5.52	0.03	0.5

There is no direct method, aside from the gravimetric, for measuring plasma protein and consequently there is no standard method against which the accuracy of another method may be tested. However, a few determinations were made by the method described and also by the Kjeldahl method, which showed an agreement to within about 5 per cent in all but one instance. The results are shown in Table V.

The method for plasma proteins here described has the advantages of being direct, simple and of requiring very little time on the part of the analyst, although the final results cannot be obtained in less than 1 or 2 days. It requires very small quantities of plasma and is accurate to within 0.3 gm. per cent, which is quite adequate for clinical work.

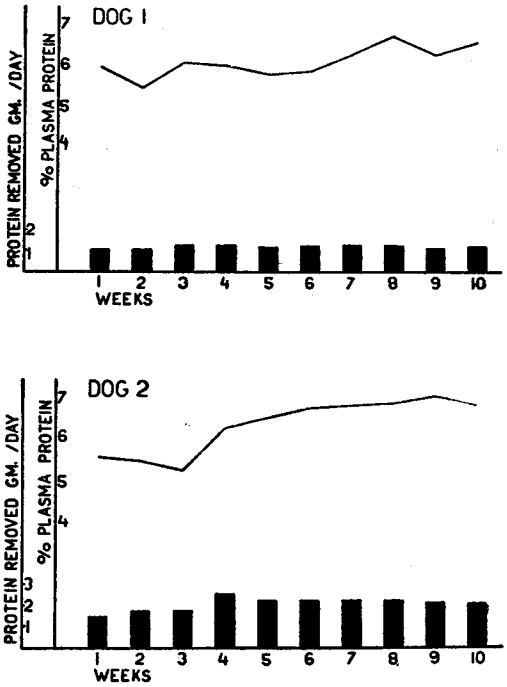

CHART 1. The effect on the plasma protein concentration of the daily loss of protein by repeated bleeding in dogs.

EXPERIMENTAL DATA

Experiments were carried out on five dogs weighing between 10 and 15 kilos. The amounts of blood removed daily varied from 40 to 150 cc. In Dogs 1 and 2, the hemoglobin did not fall appreciably, but in Dogs 3, 4 and 5 it fell steadily to about 50 per cent within the first 2 weeks and remained at approximately this level throughout the experiment. This fall in hemoglobin resulted in a relative increase in

the amounts of plasma and hence of protein that could be removed as the experiment progressed. In each case the plasma protein fell slightly for the first 2 to 4 weeks and then increased to slightly more than the initial value by the end of the experiment. The results are shown graphically in Charts 1 and 2 and are summarized in Table VI. For the sake of brevity and to eliminate minor daily variations, the results are all expressed in weekly averages. Only total plasma proteins were measured.

All the dogs were on a liberal meat diet and no attempt was made to control it quantitatively, nor to determine the effect of diet on protein regeneration.

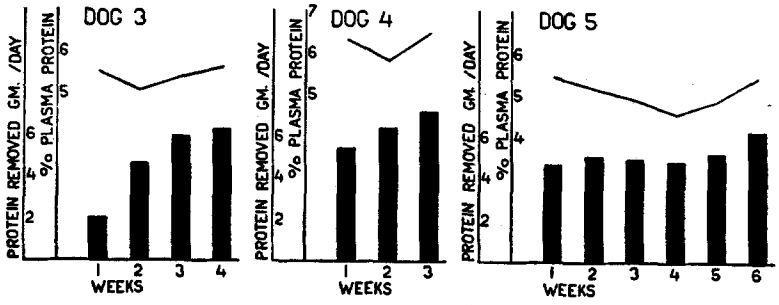

CHART 2. The effect on the plasma protein concentration of the daily loss of protein by repeated bleeding in dogs.

Dogs 1 and 2 remained in excellent condition throughout the experiment. The experiments on Dogs 3, 4 and 5 were terminated by the deaths of the animals. Autopsies were performed in each case but no cause of death was found beyond mild bronchopneumonia. Postmortem blood cultures taken in one case showed no growth. Experiments were started on three more dogs with the daily removal of 150, 200 and 250 cc. of blood but death occurred in all three before sufficient time had elapsed for the collection of significant data.

The rate of regeneration of plasma proteins in man has not been measured because of the danger of the removal of large amounts of blood proteins. In patients with kidney disease, large amounts of protein are often lost in the urine but this is usually accompanied by low plasma proteins, which may result either from the protein loss or

from failure of regeneration. We recently had a patient with cirrhosis of the liver who required frequent abdominal tapping and whose ascitic fluid contained considerable amounts of protein. This protein

TABLE VI

Effect on the Plasma Protein of the Daily Loss of Protein from Repeated Bleedings in Dogs

Dog	Weight	Average daily removal			Plasma protein		Duration of experiment
		Blood	Protein		Average 1st wk.	Average last wk.	
	kg.	*cc.*	*gm.*	*gm./kg.*	*per cent*	*per cent*	*wks.*
1	14.1	40	1.2	0.09	5.9	6.6	10
2	11.1	60	2.2	0.20	5.6	6.9	10
3	10.8	150	4.9	0.45	5.5	5.6	4
4	13.4	150	6.3	0.47	6.3	6.5	3
5	12.8	150	5.2	0.41	5.5	5.4	6

TABLE VII

Effect on the Plasma Protein of Loss of Protein in Ascitic Fluid

Date	Amount of fluid	Protein content	Protein lost	Protein lost	Plasma protein
	liters	*per cent*	*gm.*	*gm./day*	*per cent*
12/29/30	10.0	1.10	110	5.8	5.6
1/20/31	8.5	1.49	127	5.8	6.7
2/ 6/31	11.0	1.07	118	6.9	5.9
2/26/31	9.5	1.00	95	4.7	6.9
3/12/31	11.7	1.30	152	10.8	
3/28/31	11.7	1.37	160	10.0	6.1
4/11/31	12.5	1.22	152	10.9	5.9
4/28/31	14.0	1.60	224	13.2	5.5
5/16/31	13.1	1.63	214	11.9	
6/ 1/31	13.1	1.46	191	12.0	5.8
6/18/31	13.5	1.42	192	11.3	5.1
7/ 3/31	12.4	1.41	175	11.7	4.9
7/21/31	13.0	1.37	178	9.9	5.2

presumably was derived from the blood proteins and since there was no significant drop in the level of these, he was apparently able to regenerate sufficient to make up for the loss. A summary of the tappings and plasma proteins in this case is presented in Table VII.

At the end of the period of observation, this patient had produced 206 liters of ascitic fluid. Shortly after the last tapping a large umbilical hernia broke down allowing the constant escape of fluid and making further quantitative observations impossible. His urine was tested frequently and showed no significant abnormalities. His urinary protein excretion was never above the normal limits. The total period of observation was 204 days, during which he excreted a total of 2088 gm. of protein, an average of 10.2 gm. per day. His plasma proteins showed a good deal of variation during this time but there was no definite fall.

DISCUSSION

In all of the experiments described the level of the plasma proteins was maintained in spite of the daily removal of considerable quantities of protein. The duration of most of the experiments was sufficiently long to rule out the possibility of an emergency storage of protein for the purpose of maintaining this level, and it is assumed that regeneration was complete and sufficiently rapid to make up for the loss. There is a possibility that the concentration of the plasma proteins was maintained at the expense of a decrease in the total amount of circulating protein. In Dog 5, the plasma volume was measured by the method of Hooper, Smith, Belt and Whipple (10) at the beginning and at the end of the 4th week of the experiment. The initial plasma volume was 630 cc. and at the end of the 4th week it was 600 cc. These results agree within the limits of error of the method and do not indicate any decrease in the total amount of plasma.

In spite of the loss of widely varying amounts of protein, the constancy of the plasma protein levels in each individual dog is quite striking. In all cases except in Dog 2 in which it was slightly higher, the final level was almost identical with the initial one. The early fall with the subsequent rise to the original value suggest that the mechanism for regeneration requires some time for its complete development.

The regeneration of the plasma proteins can apparently be quite rapid and can be maintained over a long period of time. The daily regeneration in our experiments was of about the same magnitude as that observed by Smith, Belt and Whipple (2) but was maintained for

a period of 6 weeks in Dog 5 instead of from 2 to 7 days. That regeneration can be still more rapid is suggested by an experiment of Leiter (6) in which nearly a liter of blood was removed daily from a dog for 28 days and by one of Shelburne and Egloff (8) in which 13 liters of plasma were removed in the course of 37 days. In both of these experiments, the level of the blood protein dropped to extremely low values but, although the actual amount regenerated cannot be measured, it must have been tremendous.

These results indicate that the loss of considerable quantities of protein is not alone sufficient to produce lowering of the level of plasma protein and they suggest that in nephritis of the degenerative type, there may be an associated interference with the mechanism of regeneration. Linder, Lundsgaard and Van Slyke (11) conclude that protein loss is not sufficient to explain the low blood proteins in nephritis, although they observed low plasma proteins whenever the urinary protein excretion was above about 1 gm. for 24 hours. The daily loss of more than 10 gm. of protein over a period of 7 months in our case of cirrhosis had no appreciable effect on the concentration of plasma proteins.

SUMMARY

1. Experiments on five dogs are described consisting in the daily removal of blood plasma in amount from 25 to 100 cc. the red cells being returned to the circulation in Locke's solution. In no case was there a significant drop in plasma protein concentration.

2. A gravimetric method for the determination of total plasma protein is described.

3. A case is reported of cirrhosis of the liver in which over 10 gm. of protein daily was lost in the ascitic fluid during a period of 7 months without any lowering of plasma protein concentration.

4. The constancy of the plasma protein level and the adequacy of the mechanism of regeneration is pointed out.

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